Original Article

Effects of Maternal Alpha Methyldopa Administration on Memory of Rat Offspring during Growing Age

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ABSTRACT

Background: Alpha Methyldopa (AMD) is a well-known treatment for the pregnancy induced hypertension and commonly used in several countries. Indeed the possible effect of AMD on the behavioral activities of offspring, whom are exposed during fetus period, has not been studied. The present study evaluated the possible effect of maternal administration of AMD on the rat offspring memory in the growing age.

Methods: This study was carried out in Isfahan Faculty Of Pharmacy in 2015. Pregnant wistar rats were injected 400 mg/kg AMD or saline every day from 14^{th} to 21^{st} pregnancy period, respective to their group (n=8). The spatial memory of male offspring (n=9) was evaluated one, two and three month after the birth in the object recognition task. Also two groups of adult animals (n=7) were daily administered 400 mg/kg AMD or saline one week prior to the memory evaluation. The discrimination (d2), recognition (R) and frequencies of exploration of new object (F_B) in the T2 trials are used as the memory indicating factors.

Results: Daily single dose of 400 mg/kg AMD to the mothers one week prior to the delivery significantly decreased the d2 index, R index and F_B in two and three months offspring rats compare to the their respective control groups.

Conclusion: The newborn rats exposed maternally to the AMD during the fetus period show cognitive impairments in the growing age. Indeed the rate of memory enhancement follows a slow pattern compare to the control offspring rats.

KeyWords: Alpha Methyldopa, Memory, Pregnancy, Offspring, Rat.

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INTRODUCTION

Alpha-Methyldopa (AMD) is a classic antihypertensive agent used in the treatment of hypertensive crisis [1]. In some countries, including United States and United Kingdom, the AMD is the first line on the treatment of pregnancy hypertension and commonly used in the pregnant women [2, 3]. A great part of the antihypertensive effect of AMD is related to its stimulation of the presynaptic alpha two adrenergic receptors [4]. Alpha two receptors are found in different regions of the central and peripheral nervous systems. The stimulation of alpha 2 adrenergic receptor will inhibit the further release of different neurotransmitters like serotonin, norepinephrine and dopamine [5-7].

In addition, AMD can disrupt the synthesis process of norepinephrine and dopamine to produce an inefficient mis-metabolite called alpha methyl norepinephrine and alpha methyl dopamine [7, 8]. AMD alters serotonin (5HT) synthesis, storage and release in the central nervous system [5].The above neurotransmitters play an important role in the synaptic transmission process like memory and cognitive functions [9-11]. Some brain regions such as the prefrontal cortex, hippocampal formation and corpus striatum, which strongly involved in cognition, are densely innervated by serotonergic and dopaminergic afferents from the raphe complex and the meso-cortico-limbic or nigrostriatal systems, respectively [9].

Based on the easily entrance of AMD to the fetus, as well as its ability to affect the cognitive related modulators [9-12], it seems AMD possesses a capacity to change the behavioral process like memory [13].

Although AMD is commonly used to alleviate the pregnancy induced hypertension, but its possible effects on the behavioral process of young and adult offspring has not been well studied.

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The current study was designed to evaluate the memory of AMD maternally exposed offspring rats in the first postpartum months by the object recognition task (ORT).

MATERIALS AND METHODS

Animals

The experiments were carried out on the mature female wistar rats (230±20) obtained from the Animal House of Isfahan Pharmacy School. The animals had free access to food and drinking water during the experiment and were kept at a constant room temperature (25±5 °C), under a 12-h light/dark cycle. The experiments were conducted in the light phase of the cycle.

All procedures were reviewed and approved by the Animal Care Committee in Isfahan University of Medical Sciences.

Drugs

Alpha Methyldopa (levo-3-(3, 4-dihydroxyphenyl) - 2-methylalanine sesquihydrate $C_{10}H_{13}NO_4$ Fluka (1426002)) was purchased from Zahravi Company in Iran. The scopolamine was prepared from Daru Pakhsh Company in Iran. The solutions of drugs were prepared freshly in saline every day.

Drug Administration

Each eight female rats were housed with a male rat in each group for breeding. The pregnancies were confirmed by an expert technician. The pregnant animals were divided randomly to two groups. In one group, animals were daily-injected 400 mg/kg AMD intraperitoneally, from 14^{th} to 21^{st} days of pregnancy that is the last trimester of rat pregnancy. Other group of animals received the same volume of saline for the similar period.

After the completion of feeding period, the male offspring in treatment or control group (n=9) were separated and are tested for the spatial memory one, two and three months after the birth via ORT. Besides, in two groups (n=7), adult three months male rats were daily injected AMD or saline respectively for 7 d one week prior to the ORT procedure. As a positive control a group of rats (n=7) were injected 0.5 mg/kg of scopolamine every day for one week.

Apparatus and Objects

The ORT apparatus consist of a circular arena 83 cm in diameter and 40 cm high wall was made from white polyvinyl chloride. Two different sets of objects consist of a massive aluminum cube (10×5×7.5 cm) and a tapering top (13×8×8cm). Each object was available in triplicate. The objects could not be displaced by rats.

Experimental Procedures

The animals were transferred to the arena for adaptation procedure. For this purpose, they were allowed to explore the apparatus (without any objects) twice in a 5 min period with 1 h interval one day before the memory-testing day.

Object recognition consists of three clearly defined phases: a training session or first trial (T1), a one-hour training–test interval, and a test session or second trial (T2). Each trial lasted 5 min. During the T1, each rat was placed into the arena and exposed to two identical objects (A1 and A2) for a period lasting 5 min. Two objects were placed in a symmetrical position about 10 cm away from the wall. The rats were then returned to their home cage for a 1 h inter-trial interval. The entire arena was cleaned with alcohol (70%), both objects removed and one replaced with an identical familiar copy and one with a novel object. Then rats were returned to explore the familiar (A) and novel object (B) in the T2.

Exploration was defined as follows: directing the nose to the object at a distance of no more than 2 cm and/or touching the object with the nose. Sitting on the object was not considered exploratory behavior. The exploration time (s) for each object in each trial was recorded and the following factors were calculated

e_1: The total exploration time of both objects in the first trial ($eA_1 + eA_2$)

e_2: The total exploration time of both objects in the second trial (eA + eB)

d_2: discrimination index (eB - eA)/ (eB + eA)

d_2 is an index indicates the discrimination between the new and the familiar objects. Its value vary between +1 and -1, where a positive score indicates more time spent with the novel object, a negative score indicates more time spent with the familiar object, and a zero score indicates a null preference.

F= Frequency of the object exploration

Recognition Index (RI): [RI = eB/ (eB + eA)]

Other measures of the object recognition task is RI which is the time spent to explore the novel object relative to the both objects exploration time [14].

Statistical Analysis

Results are expressed as mean± SEM. The data value of different factors indicating the memory was analyzed using one-way analysis of variance (ANOVA). For multiple comparisons, The Tukey post hoc tests were used. *P*-values less than 0.05 were considered statistically significant.

RESULTS

The Effect of Prenatal Administration of Alpha Methyldopa on the D2 Index and R Index in One, Two and Three Month/S Offspring Rats

Prenatal injection of 400 mg/kg AMD for one week from 14th to 21st days of pregnancy significantly reduced the d2 index and R index of T2 trial in offspring after two ($P<0.05$, $P<0.05$) or three ($P<0.01$, $P<0.05$ respectively) months from the birth compare to their respective control groups. The reduction in d2 index was more remarkable in the three months offspring rat in comparison to their control group. There were no statistically differences in d2 index or R index between the control and prenatal AMD treated offspring, one month after the birth. AMD injection in the adult three months rats for one week did not show significantly reduction in the d2 or R index compare to their control group (Figure1, 2).

The Effect of Prenatal Injection of Alpha Methyldopa on the Frequency of New Object Exploration in the T2 Trial in One, Two and Three Month/S Offspring Rats

Although prenatal AMD did not change the frequency of exploration in one month offspring, it significantly was reduced two ($P<0.05$) and three ($P<0.05$) months after the birth. There was no difference in frequency of new object exploration between the control and AMD treated adult rats (Figure 3).

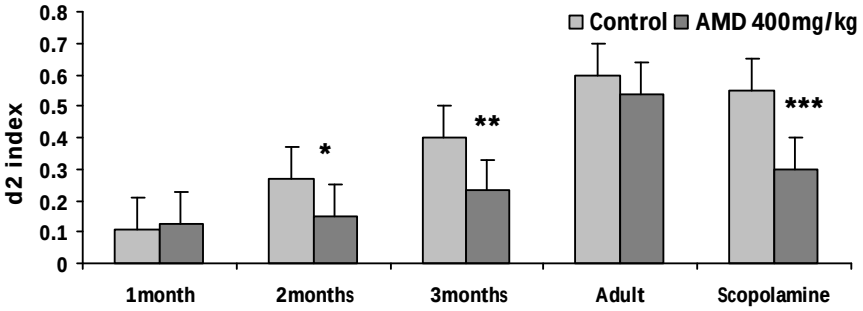

Figure 1. The comparative effect of prenatally exposure to alpha methyldopa in one, two and three month/s offspring rats on the d2 index inT2 trial.

Prenatal AMD significantly reduced the d2 index in offspring rat after two ($P<0.05$) or three ($P<0.01$) months from the birth. There is no statistically difference in one-month offspring or adult 3 months rats compare to their respective control. Data are presented as Mean±SEM. (*$P<0.05$, **$P<0.01$) (d2: discrimination, AMD:Alpha methyldopa).

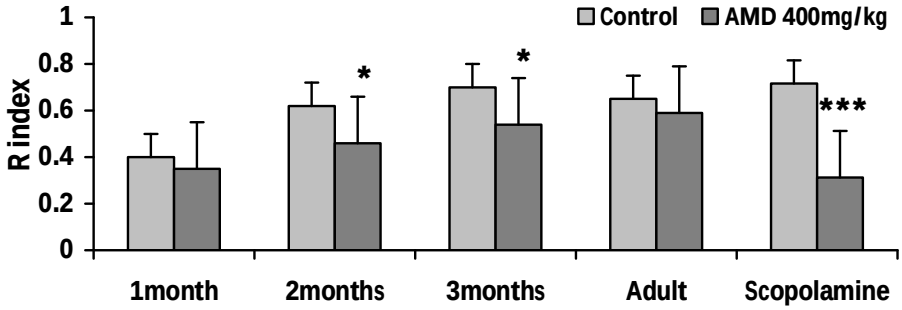

Figure 2. The comparative effect of prenatally exposure to alpha methyldopa in one, two and three month/s offspring rats on the R index inT2 trial.

Prenatal AMD significantly reduced the R index of T2 trial in offspring rat after two ($P<0.05$) or three ($P<0.05$) months from the birth. There is no statistically difference in one-month offspring and adult 3 months rats compare to control. Data are presented as Mean±SEM. (*$P<0.05$) (R: Recognition, AMD:Alpha methyldopa)

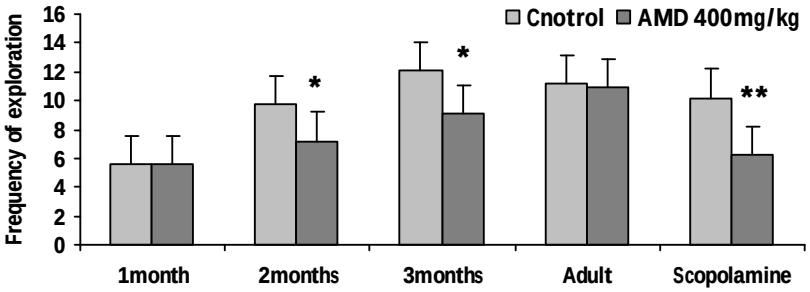

Figure 3. The comparative effect of prenatal injection of alpha methyldopa on the frequency of new object exploration in the T2 trial in one, two and three month/s offspring rats.

Although prenatal AMD did not change the frequency of exploration in one month offspring and adult 3 months rats , it significantly was reduced two(P<0.05) and three(P<0.05) months after the birth. Data are presented as Mean±SEM. (*P<0.05) (F(A): Frequency of exploration for familiar object. F(B): Frequency of exploration for new object, AMD:Alpha methyldopa)

DISCUSSION

The current study was performed to evaluate the effect of prenatal exposure to the alpha methyldopa, a classic anti-hypertensive drug severally used in pregnant mother, on the memory of children during growing age in the rat [1- 3]. For the assessment of memory we used the object recognition task which is a stress-less and reliable task for the evaluation of spatial memory [15, 16].

We found that the administration of AMD during the pregnancy period from 14th to 21st days could decrease the memory evaluating factors like d2, R and F index, two and three months after the birth in their offspring. These data show prenatal AMD alters the growing rate of memory in the offspring during the developing age. We did not see any difference in the memory indicating factors in one-month age offspring compare to their control. In addition, one-week treatment of to the adult rats did not change their memory profile in the ORT. Interestingly, the injection of scopolamine, as a well-known memory destructive agent, significantly decreased the memory assessing factors [17, 18]. This indicates the ORT is able to define the memory changes induces by AMD in the study.

AMD can easily pass the placenta and find a measurable level in the fetus [12]. In addition, it can freely access the brain to alleviate the hypertension [19]. AMD belongs to the alpha two adrenergic receptor stimulants that treat the hypertension based on this mechanism [4]. Stimulation of alpha two receptor decreases the release of several neurotransmitter like norepinephrine [6], dopamine [7], serotonin [5] and acetylcholine [20, 21]. The mentioned neurotransmitters play an important role in the formation of memory [9-11]. This mechanism could describe, to some extent, the observed cognitive impairment in the offspring. AMD also could involve the norepinephrine (NEP) and dopamine formation pathway lead to the production of a false metabolite [7, 8]. It can change the physiologic processes related to the NEP or dopamine e.g. learning and memory [9, 10].

In addition, it may explain why AMD can inhibit intellectual progress in infants. According to the present results, one-week administration of AMD is not enough to change the memory in adults but it can change the memory in the offspring. Indeed, AMD may disrupt the anatomical or physiological processes in the fetus that it can caused memory permanent deterioration in the growing age. We did not find any difference in the memory for one-month rat related to their control. The ORT arena for rat is not perfect method to evaluate the memory-indicating factor for the body of one-month rat. On the other hand, it may be too soon to find the probable difference between one-month rat and control. In addition, the neuronal processes are involved in the memory formation are more susceptible to the dopamine disruption in the prenatal stages. Conversely, there are some literatures showed the improving effects of alpha2 stimulation on the memory impairment in aged rats [22, 23].

CONCLUSION

Memory formation is a complex process that requires distinct neuronal networks and multiple pre- and postsynaptic events. Several studies show that different neurotransmitter in the central nervous system play a key role in the learning and memory. Administration of AMD in

the pregnancy period, affect the memory process of offspring evaluated two and three months after the birth in ORT. It cannot change the memory of adult rat when is injected three month after the birth.

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